GUIDELINES ON THE USE OF MASS SPECTROMETRY (MS) FOR IDENTIFICATION, CONFIRMATION AND QUANTITATIVE DETERMINATION OF RESIDUES

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CONFIRMATORY TESTS

When analyses are performed for monitoring or enforcement purposes, it is particularly important that confirmatory data are generated before reporting on samples containing residues of pesticides that are not normally associated with that commodity, or where MRLs appear to have been exceeded. Samples may contain interfering chemicals that may be misidentified as pesticides. Examples in gas chromatography include the responses of electron-capture detectors to phthalate esters and of phosphorus-selective detectors to compounds containing sulphur and nitrogen.

Analysis of pesticide residues with multi-residue methods generally consists of two phases: screening and confirmation. The process is schematically depicted in Fig. 2. The first phase comprises establishment of those pesticide residues that are likely to be present from interpreting the raw data, avoiding false negatives as much as possible. The second phase is the confirmation, which focuses on the pesticides found in phase 1. The use of the results to be reported, and consequent management decision determines the efforts put in the confirmatory process. The choice of the technique used for confirmation depends on their availability, time and cost. They are based on either further interpretation of chromatographic and mass spectrometric data, alternative methods using different physico-chemical properties of the compound, or a combination of various separation and detection methods. Some alternative procedures for confirmation are given in Table 6.

Whenever chromatographic techniques are used in screening or confirmation, proper settings of the retention time windows is pivotal. Care should be taken that the instrument is adjusted correctly before starting the analysis; a system suitability test should be performed prior to each batch of analysis[1]. Retention times data base should be adjusted for the current conditions[2]. In phase 1, tolerance intervals of 1.5 to 3% of the absolute retention time may be applied for capillary GC depending on the peak shape. For confirmation of the retention time, the absolute tolerance intervals will increase at higher retention time. The tolerance interval should be less than 1 sec for an RT less than 500 sec. For retention times between 500 and 5000 sec. an interval of 0.2% RRT is recommended. For higher retention times 6 sec. is a suitable interval.

Confirmatory tests may be quantitative and/or qualitative but, in most cases, both types of information will be required. Particular problems occur when residues must be confirmed at or about the limit of determination, although it is difficult to quantify residues at this level, it is essential to provide adequate confirmation of both level and identity.

The need for confirmatory tests may depend upon the type of sample or its known history. In some crops or commodities, certain residues are frequently found. For a series of samples of similar origin, which contain residues of the same pesticide, it may be sufficient to confirm the identity of residues in a small proportion of the samples selected randomly. Similarly, when it is known that a particular pesticide has been applied to the sample material, there may be little need for confirmation of identity, although a number of randomly selected results should be confirmed. Where "blank" samples are available, these shall be used to check the occurrence of possible interfering substances.

The necessary steps for positive identification are a matter of judgement on the analyst's part, and particular attention should be paid to the choice of a method that would minimise the effect of interfering compounds.

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[1] Soboleva E. Ambrus A., Application of system suitability test for quality assurance and performance optimization of a gas chromatographic system for pesticide residue analysis, J. Chromatogr. A. 1027. 2004. 55-65.

[2] Lantos J., Kadenczki L., Zakar F., Ambrus A. Validation of gas chromatographic Databases for qualitative identification of active ingredients of pesticide residues in Fajgelj A. Ambrus A. (eds) Principles of Method Validation, Royal Society of Chemistry, Cambridge, 2000, pp 128-137.

The technique(s) chosen depend(s) upon the availability of suitable instruments and expertise within the testing laboratory.

GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS)

Residue data obtained using mass spectrometry represents the most definitive evidence and, where suitable instrumentation is available, it is the confirmatory technique of choice. The technique is also used commonly for residue screening purposes (phase 1). Mass spectrometric determination of residues is usually carried out in conjunction with a chromatographic separation technique to provide retention time ion mass/charge ratio and abundance data simultaneously. Quantitative transmission of labile analytes through the chromatographic system is subject to problems similar to those experienced with other detectors. For quantification, the ions monitored should be those that are the most specific to the analyte, are subject to least interference and provide good signal-to-noise ratio.

When using selected ion monitoring (SIM), tolerance intervals of ion ratios and retention times based on injection of pesticide standard in pure solvent at the concentration close to the critical level should have been established at this point. The tolerance intervals for the ion ratios should be within the limits of ± 30 % of absolute ion abundances ratios. When 2 (or 3) selected ion ratios are within the established tolerance intervals the residue is confirmed[3]. For a small number of pesticides the mass spectrum may only exhibit one specific ion. In this case alternative confirmation should be sought.

When the ions detected still indicate the possible presence of a residue, the result may be reported as tentatively identified. However, when the result would lead to regulatory action, or results would be used for other purposes (e.g. dietary intake assessment) further confirmation of analyte identity shall be sought. This can be achieved with the same GC-MS instrumentation, by injecting matrix-matched standards of the suspected analyte, in order to compensate for matrix influence on ion ratios. In this case, subsequent injections of matrix matched standard and suspected sample has to be made. The deviation of RRT of analyte in standard and suspected peak in sample should typically be less than 0.1 %. Two ion ratios measured in a sample should be within the tolerance interval calculated based on the ion ratios in matrix-matched standard. The residue is considered to be confirmed if it complies with the general rule stated above. If the ion ratios are not within the tolerance intervals, additional confirmation of identity may be obtained by the use of alternative analytical techniques. Examples are listed in Table 6.

Further confirmation by mass spectrometry can be accomplished by acquisition of the complete electron-impact mass spectrum (in practice generally from m/z50 to beyond the molecular ion region). The absence of interfering ions is an important consideration in confirming identity. Additional confirmation of identity may be obtained by (i) the use of an alternative chromatographic column; (ii) by the use of an alternative ionisation technique (e.g. chemical ionization); (iii) by monitoring further reaction products of selected ions by tandem mass spectrometry (MS/MS or MS^n); or (iv) by monitoring selected ions at increased mass resolution.

Mass spectrometric determinations should satisfy similar analytical quality control criteria to those applied to other systems.

HPLC AND HPLC-MS

Confirmation of residues detected following separation by HPLC is generally more problematic than where gas chromatography is used. If detection is by UV absorption, production of a complete spectrum can provide good evidence of identity. However, UV spectra of some pesticides are poorly diagnostic, being similar to those produced by many other compounds possessing similar functional groups or structures, and co-elution of interfering compounds can create additional problems. UV absorption data produced at multiple wavelengths may support or refute identification but, in general, they are not sufficiently characteristic on their own. Fluorescence data may be used to support those obtained by UV absorption.

[3] Soboleva E. Ahad K. Ambrus A. Applicability of some MS criteria for the confirmation of pesticide residues, . Analyst, 129, 1123-1129, 2004.

LC-MS can provide good supporting evidence but, because the spectra generated are generally very simple, showing little characteristic fragmentation, results produced from LC-MS are unlikely to be definitive. LC-MS/MS is a more powerful technique, combining selectivity with specificity, and often provides good evidence of identity. LC-MS techniques tend to be subject to matrix effects, especially suppression, and therefore confirmation of quantity may require the use of standard addition or isotopically-labelled standards. Derivatisation may also be used for confirmation of residues detected by HPLC (Table 6).

THIN LAYER CHROMATOGRAPHY (TLC)

In some instances, confirmation of gas chromatographic findings is most conveniently achieved by TLC. Identification is based on two criteria, Rf value and visualisation reaction. Detection methods based on bioassays (e.g. enzyme -, fungal growth or chloroplast inhibition) are especially suitable for qualitative confirmation as they are specific to certain type of compounds, sensitive and normally very little affected by the co-extracts[4,5]. The scientific literature contains numerous references to the technique[6]. The quantitative aspects of thin-layer chromatography are, however, limited. A further extension of this technique involves the removal of the area on the plate corresponding to the Rf of the compound of interest followed by elution from the layer material and further chemical or physical confirmatory analysis. A solution of the standard pesticide should always be spotted on the plate alongside the sample extract to obviate any problems of non-repeatability of Rf. Over-spotting of extract with standard pesticide can also give useful information. The advantages of thin layer chromatography are speed, low cost and applicability to heat sensitive materials; disadvantages include (usually) lower sensitivity and separation power than instrumental chromatographic detection techniques and need for more efficient cleanup in case of detections based on chemicals colour reactions.

DERIVATISATION

When selecting ions for GC/MS confirmation based on a derivative, the selected ions must be structurally significant for the residue and not represent fragments of the derivatizing agent. Whereas derivatisation might be a valuable way to confirm the identity of a residue, it should be taken into account that it will also add an extra element to the uncertainty of a quantitative confirmation .

This area of confirmation may be considered under three broad headings.

(a) Chemical reactions

Small-scale chemical reactions resulting in degradation, addition or condensation products of pesticides, followed by re-examination of the products by chromatographic techniques, have frequently been used. The reactions result in products possessing different retention times and/or detector response from those of the parent compound. A sample of standard pesticide should be treated alongside the suspected residue so that the results from each maybe directly compared. A fortified extract should also be included to prove that the reaction has proceeded in the presence of sample material. Interference may occur where derivatives are detected by means of properties of the derivatising reagent. A review of chemical reactions which have been used for confirmatory purposes has been published by Cochrane, W.P. (Chemical derivatisation in pesticide analysis, Plenum Press, NY (1981)). Chemical reactions have the advantages of being fast and easy to carry out, but specialised reagents may need to be purchased and/or purified.

(b) Physical reactions

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[4] Ambrus[1]* Á.,. Füzesi[2] I.; Susán[2] M.; Dobi[3] D., Lantos[4] J., Zakar[5] F., Korsós[4] I., Oláh[3] J., Beke[3] B.B., and L. Katavics[5] A cost effective screening methods for pesticide residue analysis in fruits, vegetables and cereal grains, J. Environ Sci. Health B40, 297-339, 2005.

[5] Ambrus Á.; Füzesi I.; Lantos J.; Korsos I.; Hatfaludi T. Repeatability and Reproducibility of Rf and MDQ Values with Different TLC Elution and Detection Systems. J. Environ Sci. Health B39 **2004** *accepted for publication*.

[6] IUPAC Report on Pesticides (13) (Bátora, V., Vitorovic, S.Y., Thier, H.-P. and Klisenko, M.A.; Pure & Appl. Chem., 53, 1981, 1039-1049

A useful technique is the photochemical alteration of a pesticide residue to give one or more products with a reproducible chromatographic pattern. A sample of standard pesticide and fortified extract should always be treated in a similar manner. Samples containing more than one pesticide residue may give problems in the interpretation of results. In such cases pre-separation of specific residues may be carried out using TLC, HPLC or column fractionation prior to reaction.

(c) Other methods

Many pesticides are susceptible to degradation/transformation by enzymes. In contrast to normal chemical reactions, these processes are very specific and generally consist of oxidation, hydrolysis or de-alkylation. The conversion products possess different chromatographic characteristics from the parent pesticide and may be used for confirmatory purposes if compared with reaction products using standard pesticides.

Table 6. Detection methods suitable for screening (Phase 1) and confirmation (Phase 2) of residues.

		Phase 1 - Screening							
		GC with capillary column – ECD, NPD, FPD, PFPD	GC-MS	LC-MS	LC-DAD or scanning UV	LC-UV/VIS (single wavelength)	LC-fluorescence	GC with packed column – ECD, NPD, FPD	TLC – enzyme -, fungal growth or chloroplast inhibition
Phase 2, confirmation	GC – capillary column – ECD, NPD, FPD, PFPD	x[1]	x[1]	x	x	x	x	x	x
	GC-MS	x	X[1 2]	x	x	x	x	x	x
	LC-MS	x	x		x	x	x	x	x
	Full scan techniques	x	x	x	x	x	x	x	x
	$(MS)^n$, HRMS, alternative ionisation techniques	x	x	x	x	x	x	x	x
	LC-DAD or scanning UV	x	x	x		x	x	x	x
	LC-UV/VIS (single wavelength)	x	x				x	x	x
	LC-fluorescence	x	x		x	x		x	x
	TLC – enzyme, fungal growth or chloroplast inhibition	x	x	x	x	x	x	x	X[2 3]
	Derivatisation	x	x	x	x	x	x	x	x
	Specific isomers profile	x	x	x	x	x	x	x	

1 – Either the column of different polarity, which results in different elution order of the residues and contaminants eluting in the vicinity to the peak of interest, or another specific detector shell be used.

2- The same GC-MS technique can be used for the phase 2 (confirmation) if different ions are selected or tolerance intervals are established based on matrix matched solutions.

3 – Mobile or stationary phase of different polarity shall be used.

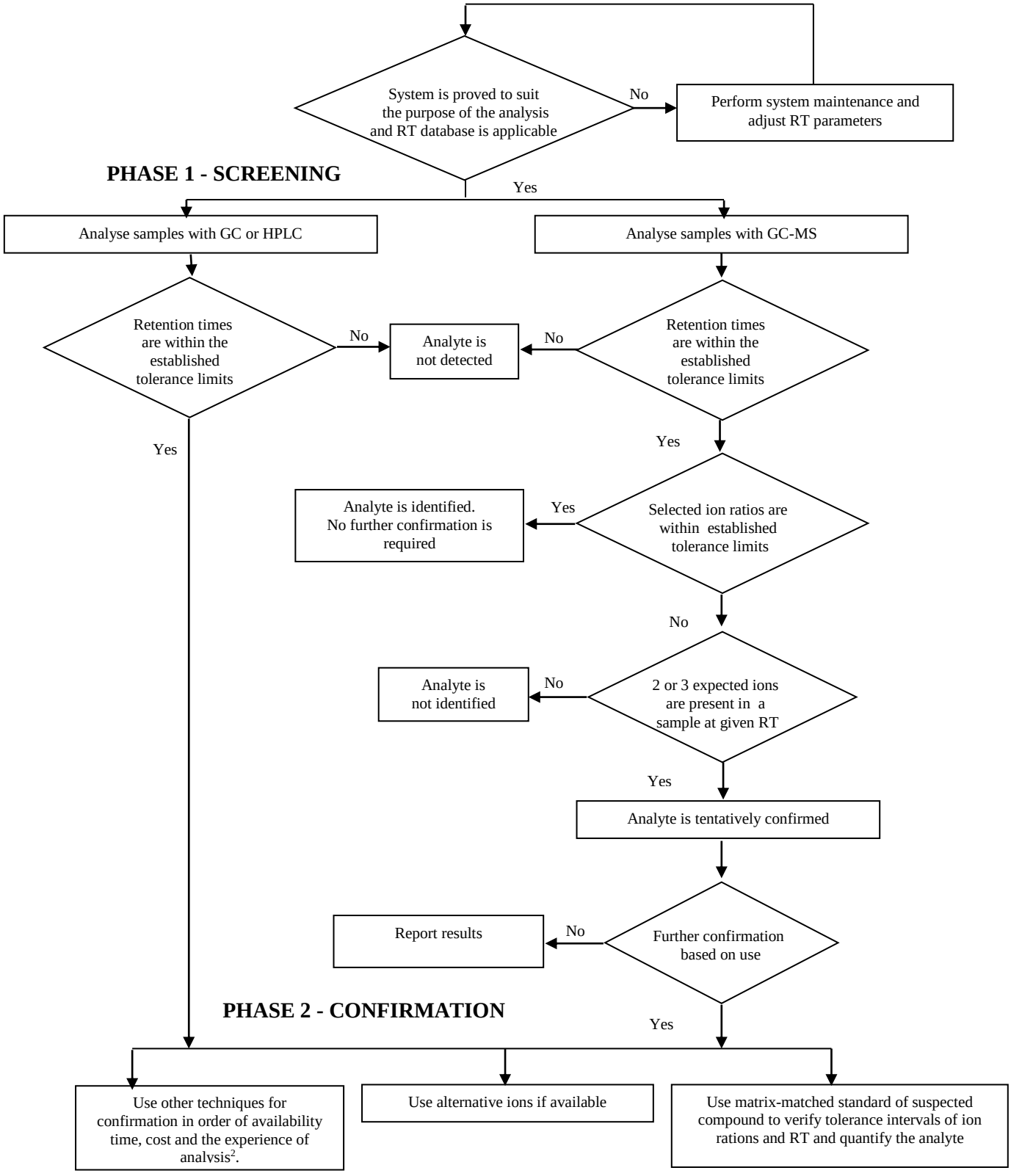

Figure 2. Schematic Representation of Screening and Confirmation (Phase 1 and Phase 2) for Pesticide Residues

1 - Unusual values including banned substances, MRL violation or study requirements as in e.g. exposure assessment

2 – Refer to table 6 for other means of confirmation

3 - For a small number of pesticides the mass spectrum may only exhibit one specific ion. In this case alternative confirmation should be sought.